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*Published in:*  
Physiological Measurement

*DOI:*  
[10.1088/1361-6579/38/3/489](https://doi.org/10.1088/1361-6579/38/3/489)

*Publication date:*  
2017

*Document Version*  
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

### *Citation for published version (APA):*

Zhao, L., Zhou, Y., Song, C., Wang, Z., & Cuschieri, A. (2017). Predicting burst pressure of radiofrequency-induced colorectal anastomosis by bio-impedance measurement. *Physiological Measurement*, 38(3), 489-500. <https://doi.org/10.1088/1361-6579/38/3/489>

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# Predicting burst pressure of radiofrequency-induced colorectal anastomosis by bio-impedance measurement

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## Abstract

The present study investigates the relationship between bio-impedance and burst pressure of colorectal anastomosis created by radiofrequency (RF)-induced tissue fusion. Colorectal anastomosis were created with ex-vivo porcine colorectal segments, during which 5 levels of compression pressure were applied by a custom-made bipolar prototype, with 5 replicate experiments at each compression pressure. Instant anastomotic tensile strength was assessed by burst pressure. Bio-impedance of fused tissue was measured by Impedance Analyzer across frequency that 100Hz to 3MHz. Statistical analysis shows only a weak correlation between bio-impedance modulus and burst pressures at frequency of 445 kHz ( $\rho_s = -0.426$ ,  $P = 0.099 > 0.05$ ). In contrast, results demonstrated a highly significant negative correlation between reactance modulus and burst pressures ( $\rho_s = -0.812$ ,  $P = 0.000 < 0.05$ ). The decrease in mean reactance modulus with increasing burst pressures was highly significant ( $p = 0.019 < 0.05$ ). The observed strong negative correlation between reactance modulus and burst pressures at frequency of 445 kHz indicates that reactance is likely to be a good index for tensile strength of RF-induced colorectal anastomosis, and should be considered for inclusion in a feedback loops in devices design.

Keywords: Radiofrequency, colorectal anastomosis, burst pressure, bio-impedance, reactance

## 1. Introduction

The feasibility of bipolar radiofrequency (RF) induced colorectal anastomosis has been confirmed by previous ex-vivo and in-vivo experiments (Smulders *et al.*, 2007; Winter *et al.*, 2010; Holmer *et al.*, 2011; Zhao *et al.*, 2013; Zhao *et al.*, 2015). Tissue fusion between approximated bowel loops is achieved by application of an alternating high frequency current for a few seconds which creates a bio-tissue weld. Unlike established traditional methods for creating colorectal anastomosis (suturing or stapling), thermo-fusion joins the colorectal walls directly without any implant. This has the potential, though not presently confirmed, of decreasing the risk of complications due to foreign materials (Hawley, 1973). Additionally, the burst pressure reflecting fusion strength is demonstrated to be equivalent to stapled anastomosed (Winter *et al.*, 2010).

There has been considerable research and design interest in recent years directed at technologies for creating strong fusion strength with minimal thermal damage to enhance safety of thermo-fused anastomosis, as excessive heating during the RF-weld causes necrosis and leakage. To prevent this, Winter and Holmer reported a closed-loop temperature-controlled bipolar RF-induced device for creation of colorectal anastomosis (Winter *et al.*, 2010; Holmer *et al.*, 2011). Another group reported on the use of infrared light detection (Floume *et al.*, 2008; Floume, 2008; Floume *et al.*, 2010) to optimize the ideal duration of the weld process during RF-induced small bowel thermo-fusion. Despite reports of effective sealing of arteries by an impedance feedback system (Kennedy *et al.*, 1998), the relationship between bio-impedance and fusion strength, remains unclear. More importantly, the bio-impedance characteristics of fused tissue have not been reported. The present study investigated these issues by experiments designed to determine relations between bio-impedance, reactance, and burst pressures of ex-vivo thermo-fused colorectal anastomosis created by a custom-made prototype bipolar RF device.

## 2. Material and methods

### 2.1. Preparation

Porcine colorectal segments, 50~60 mm in length and 2~3 mm in thick, harvested from the slaughterhouse were used after cleansing by washouts using 9% isotonic saline. The segments were then stored in chilled phosphate buffer solution (pH=7.2) until used. All thermo-fused anastomosis were performed within 6 hours after tissue harvest.

### 2.2. Experimental setup

#### 2.2.1. Compression prototype

The custom-made prototype used for creating end-to-end serosa-to-serosa colorectal anastomosis, and schematic diagram is shown in Figure 1(a) and Figure 1(b), respectively. Two electrodes were set on the electric insulated heat resistance ring carriers. The press module maintained a constant compression force (precision was 0.1 Newton) on the ring carriers during the entire fusion duration. Compression pressure per unit area of fused tissue was calculated using the formula: Compressive pressure (kPa) =  $10^{-3} \times \text{Force (N)} / [(R^2 - r^2)\pi]$  (mm<sup>2</sup>), where R and r stand for external and internal radii of the ring electrode, respectively. kPa denotes kilopascal. Five levels of compression pressure (171, 206, 242, 277, 313 kPa) were studied (Zhao *et al.*, 2015) with 5 replicate experiments at each pressure.

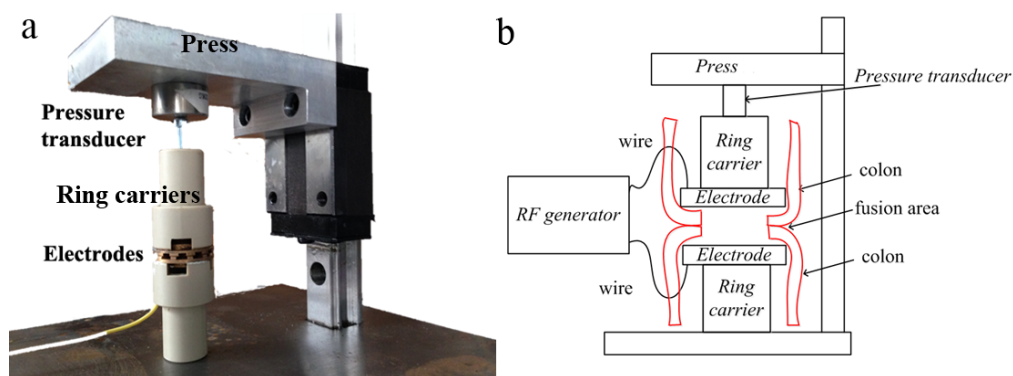


Figure 1. (a) The custom-made experimental prototype consists of press module, ring carriers, pressure transducer and electrodes. (b) Schematic diagram of end-to-end serosa-to-serosa RF-induced colorectal anastomosis performed on the experimental platform.

#### 2.2.2. Burst pressure measurement device

Burst pressures were measured by a device consisting of a T-joint pipe, pressure transducer (MB-HS, 0–0.2 MPa, Yeli Industrial Control Corporation), and injection pump (RSP01-C, Ristrion Corporation) (Figure 2). The three terminals of T-joint pipe were connected to a pressure transducer, an injection pump, and one end of fused segment. The other end of fused segment was occluded by a clamp, which

was set 50~60mm away from the stoma to avoid impact of flow velocity. During pump injection of water into the lumen at 2 ml/min(Materials, 2004), the pressure transducer transmitted a voltage signal through the data acquisition (DAQ) card (PCI6221, National Instruments Corporation) to PC and stored as pressure in mmHg. As all components were almost rigid, load effect due to elastic deformation was negligible.

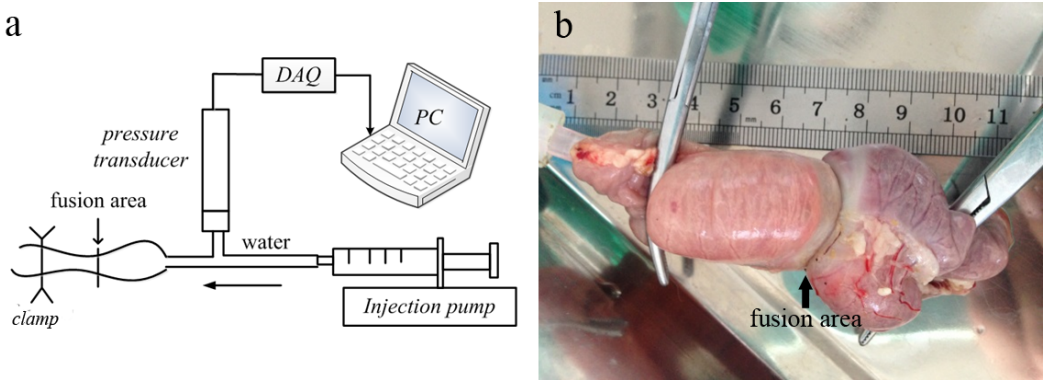


Figure 2. (a) Schematic diagram of burst pressure measurement device. (b) The injection pump injected water into lumen until fused area leaked.

### 2.2.3. Bio-impedance analyzer

Bio-impedance measurement was performed after burst pressure measurement. Test tissue was prepared as shown in Figure 3, using ‘compressed region’ which was not split by burst pressure test. This region was about 0.2 mm in thick despite distinct experimental parameters. Surface moisture was removed before testing. Figure 4(a) shows Impedance Analyzer (IM3570, HIOKI E.E. Corporation) with a 4-terminal probe (L2000, HIOKI E.E. Corporation) that the characteristic impedance is 50Ω. The normal colon was set as control. Figure 4(b) displays the ‘compressed region’ was clamped by a pair of gold-plated clip at intervals of 5 mm. When the ratio of sample size to interval of clip was extremely large, the measurement error can be ignored. Tissue was fully contacted with clips for a while before measurement to minimize influence of contact impedance. The frequency of driven signal was varied from 100 Hz to 3 MHz, by logarithmic increases. Fused samples were processed into pathological slices (3 μm in thick) for observation, which were stained with haematoxylin & eosin.

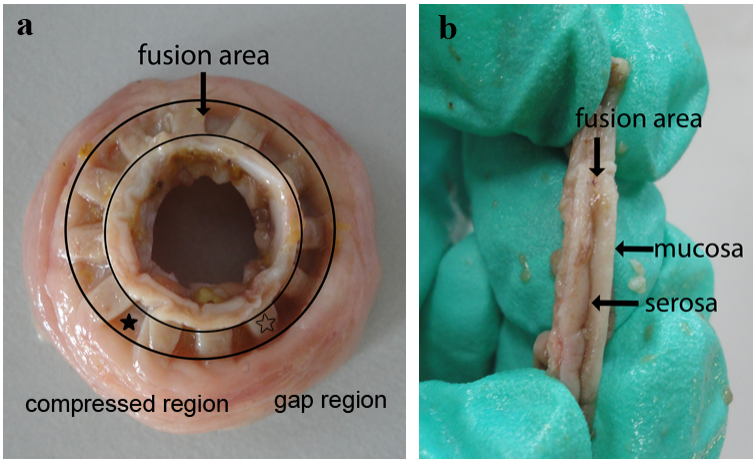


Figure 3. (a) The fused stoma is indicated by black arrow between two circles. The ‘compressed region’ and ‘gap region’ are marked by a solid star target and a blank star target, respectively. (b) The profile of fused tissue shows serosa-to-serosa welding without split.

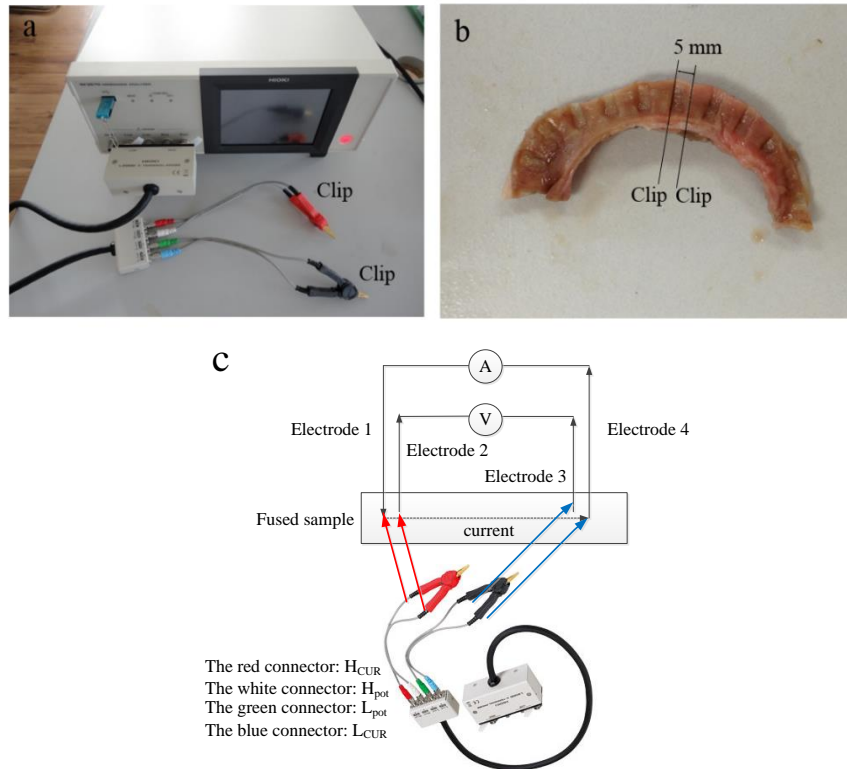


Figure 4. (a) Impedance Analyzer equipped with a couple of golden-plated clip. (b) 5 mm distance between two grasping clips. (c) The principle of impedance measurement by the 4-terminal probe.

#### 2.2.4. RF power

RF generator (Valleylab, Covidien Corporation) was used to provide electric current with frequency of 470 kHz. The power was 160 watt for welding duration of 20 seconds.

#### 2.2.5. Statistical analysis

Spearman analysis was performed (SPSS, IBM Inc.) to test the correlation between bio-impedance and burst pressures. Two groups were compared by Mann-Whitney U test. Kruskal–Wallis test was performed to analyze differences between multi-groups. Statistical significance was set at 0.05.

### 3. Results

#### 3.1. Burst pressure and histology

The burst pressures (mean  $\pm$  standard deviation) corresponding to each compression pressure are shown in Table 1. The highest burst pressure was 61.3 mmHg for 242 kPa compression pressure, with the lowest, 11.7 mmHg, obtained with compression pressure of 171 kPa. Only one colorectal anastomosis was accomplished under compression pressure that 313 kPa. The low success rate was attributed to improper compression pressure that had been discussed in other publication.

**Table 1.** Burst pressures of RF-induced porcine colorectal anastomosis underwent 5 levels of compression pressure.

Compression pressure (kPa)	Successful/ total numbers	Burst pressure (mmHg) (mean $\pm$ standard deviation)
171	4/5	30.7 $\pm$ 19.1
206	4/5	35.2 $\pm$ 20.6
242	3/5	50.2 $\pm$ 11.3
277	4/5	42.9 $\pm$ 11.2
313	1/5	24.5 <sup>a</sup>

<sup>a</sup> single value.



The histological appearances depicted in Figure 5 (a) and 5 (b) illustrate tightly and weakly fused regions, respectively.

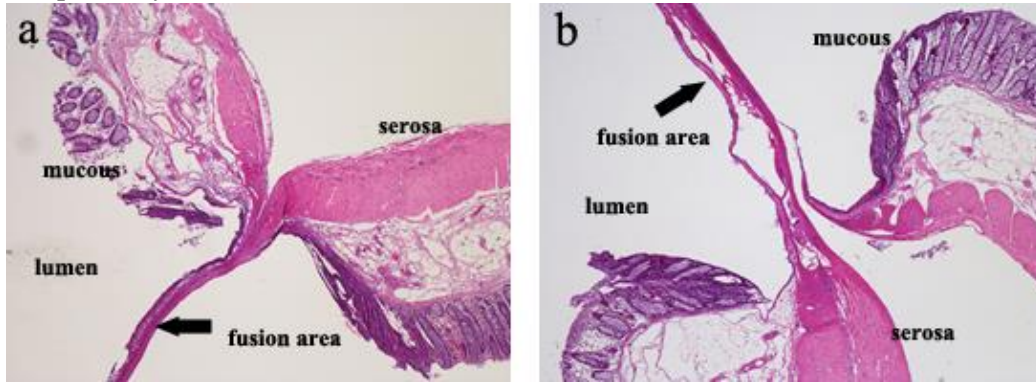


Figure 5. Transverse histological slices of fused colon (H&E, magnification  $\times 4$ ). (a) Representative slice of high burst pressure sample shows no gaps in fused area (arrow). (b) Representative slice of low burst pressure sample exhibits obvious split (arrow).

### 3.2. Bio-impedance across frequency of 1 kHz ~ 3 MHz

As interference of polarization effects occur at low frequency, data distributing on 1 kHz to 3 MHz were analyzed. Bio-impedance is a complex number consisting of resistance  $R$  and reactance  $X$  (Equation 1).  $R$  and  $X$  are calculated by Equation 2 and Equation 3, respectively (McAdams and Jossinet, 1995). For most biological tissues,  $X$  is simplified as capacitive reactance (Foster and Lukaski, 1996), ignoring inductive reactance.  $\vec{Z}$ ,  $\theta$ ,  $R$ , and  $X$  denotes complex bio-impedance, phase angle, resistance, and reactance respectively.

$$\vec{Z} = R + jX \quad (1)$$

$$R = |\vec{Z}| \times \cos \theta \quad (2)$$

$$X = |\vec{Z}| \times \sin \theta \quad (3)$$

The bio-impedance modulus were divided into five groups in terms of burst pressures. The mean bio-impedance modulus of each group versus frequency that 1 kHz to 3 MHz were illustrated in Figure 6 (a). It dropped with increasing frequency. The corresponding mean reactance modulus showed in Figure 6(b) dropped to bottom but then climbed up slightly.

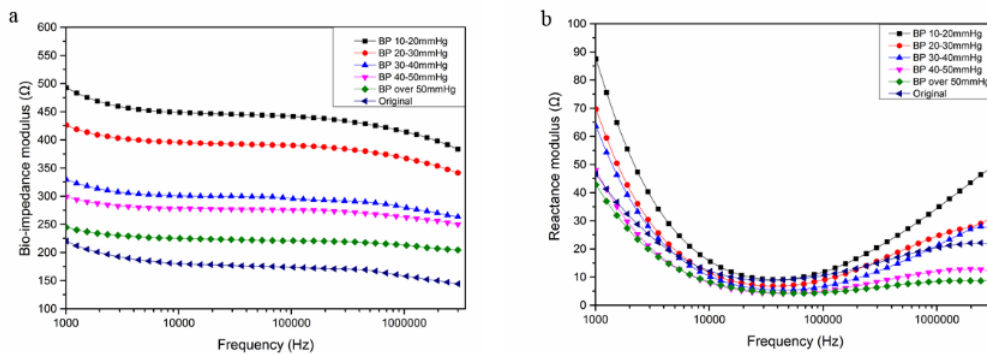


Figure 6. (a) The mean bio-impedance modulus and (b) the mean reactance modulus corresponding burst pressure levels of 10~20 mmHg (n=3), 20~30 mmHg (n=3), 30~40 mmHg (n=3), 40~50 mmHg (n=2) and over 50 mmHg (n=5) across the frequency range of 1 kHz to 3 MHz. (BP: burst pressure, Original: normal colon)

Figure 7. provides a graphic representation of the mean bio-impedance modulus across the frequency range of 150 kHz to 3 MHz categorized by five burst pressure levels. The descending of mean bio-impedance modulus with increasing burst pressure levels was not significant when analyzed by Kruskal–Wallis test ( $p=0.298>0.05$ ).

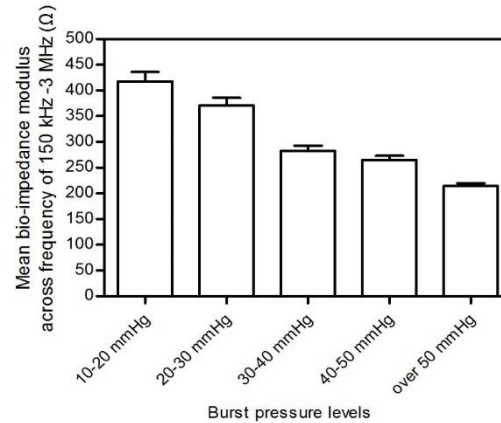


Figure 7. The mean bio-impedance modulus (mean  $\pm$  standard deviation) across frequency of 150 kHz  $\sim$  3 MHz categorized by burst pressures that 10~20 mmHg (418.2 $\pm$ 17.7 $\Omega$ , n=3), 20~30 mmHg (370.6 $\pm$ 15.1 $\Omega$ , n=3), 30~40 mmHg (282.5 $\pm$ 10.4 $\Omega$ , n=3), 40~50 mmHg (265.1 $\pm$ 8.0 $\Omega$ , n=2) and over 50 mmHg (214.2 $\pm$ 5.6 $\Omega$ , n=5).

The mean reactance modulus across frequency that 150 kHz to 3 MHz decreased with increasing burst pressure levels, showing significant differences by Kruskal–Wallis test ( $p=0.019<0.05$ ) (Figure 8). Comparing with the lowest mean reactance modulus gained from burst pressure that higher than 50 mmHg, significant difference were observed on mean reactance modulus corresponding to burst pressures of 10~20 mmHg ( $p=0.016<0.05$ ), 20~30 mmHg ( $p=0.036<0.05$ ), and 30~40 mmHg ( $p=0.036<0.05$ ).

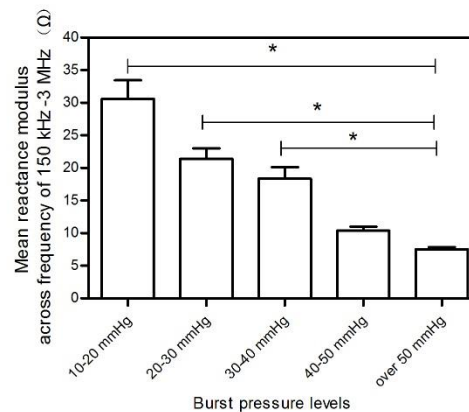


Figure 8. The mean reactance modulus (mean  $\pm$  standard deviation) across frequency that 150 kHz to 3 MHz corresponding to burst pressure levels of 10~20 mmHg (30.6 $\pm$ 11.2 $\Omega$ , n=3), 20~30 mmHg (21.4 $\pm$ 6.3 $\Omega$ , n=3), 30~40 mmHg (18.4 $\pm$ 6.9 $\Omega$ , n=3), 40~50 mmHg (10.4 $\pm$ 2.3 $\Omega$ , n=2) and over 50 mmHg (7.5 $\pm$ 1.3 $\Omega$ , n=5). \* indicates significant difference compared with burst pressure that higher than 50 mmHg on the Mann Whitney U test.

### 3.3. Bio-impedance at frequency of 445 kHz

When high-frequency electric current is applied to tissue to create fusion, the impedance of target tissue is analyzed simultaneously. Thus, the current is the power source for tissue fusion as well as the driven signal for impedance measurement. For this reason, impedance characterises at specific frequency were analyzed to predict burst pressure real-time. In present study, the frequency used by RF generator was 470 kHz, we adopted an 'approximate' frequency of 445 kHz as being closest to which. Correlation between bio-impedance and burst pressures was investigated at that frequency.

The bio-impedance modulus of fused colon ranged from 121.56  $\Omega$   $\sim$  453.32  $\Omega$  at frequency of 445 kHz. A weak negative correlation was found between bio-impedance modulus and burst pressures ( $\rho_s = -0.426$ ,  $p=0.099>0.05$ ) (Figure 9).

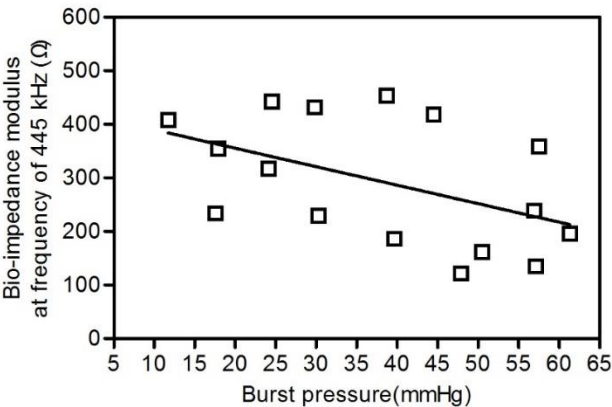


Figure 9. Weak negative correlation between bio-impedance modulus and burst pressures at frequency of 445 kHz (Spearman analysis,  $\rho_s = -0.426$ ,  $p = 0.099 > 0.05$ ).

The reactance modulus of fused colon were varied from 4.84 Ω ~ 28.35 Ω at frequency of 445 kHz. In contrast, a considerably significant negative correlation was noted between reactance modulus and burst pressures ( $\rho_s = -0.812$ ,  $p = 0.000 < 0.05$ ) (Figure 10).

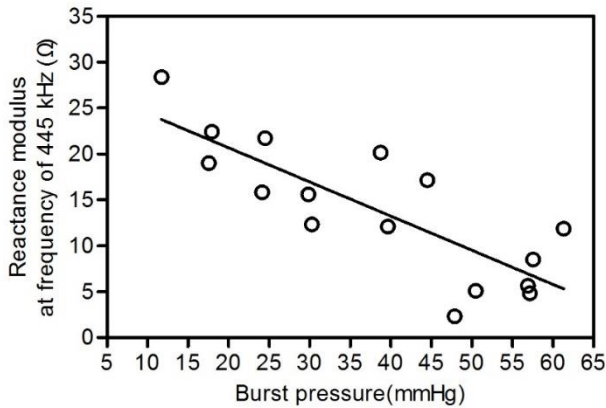


Figure 10. Significant negative correlation between reactance modulus and burst pressures at frequency of 445 kHz (Spearman analysis,  $\rho_s = -0.812$ ,  $p = 0.000 < 0.05$ ).

Furthermore, at frequency of 445 kHz, a significant difference was observed in reactance modulus corresponding to burst pressures, higher and lower than 25 mmHg ( $p = 0.003 < 0.05$ ). In contrast, no difference was noted in bio-impedance modulus ( $p = 0.108 > 0.05$ ) (Figure 11).

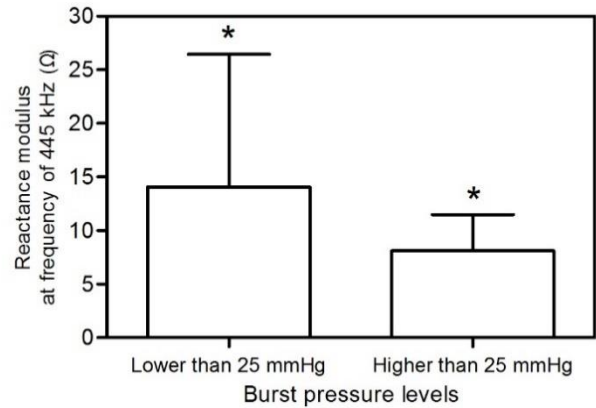


Figure 11. Reactance modulus at frequency of 445 kHz corresponding to burst pressures, higher and lower than 25 mmHg, which were significantly different on the Mann-Whitney U test (\*).



#### 4. Discussion

Anastomotic leakage due to weak fusion leading to intra-abdominal septic complications including peritonitis may result in death, and hence cannot be ignored (Hyman *et al.*, 2007). In the developed anastomotic devices for thermal fusion, burst pressure is used as an ex-vivo assessment of the strength of the fused anastomotic line, and indeed despite its limitations with respect to the actual integrity of a surgical anastomosis in-vivo, it remains the gold standard method (Materials, 2004). However there are unresolved technical issues, e.g., burst pressure measurement is influenced by the rate of water infusion (Nelsen and Anders, 1966). Thus, in a study on small bowel thermo-fused sealing a 10-mmHg increase in burst pressure was induced with increase in water inflation rate from 2 ~19 ml/min (Arya *et al.*, 2013). For this reason, Hendriks (Hendriks and Mastboom, 1990) and Cezo (Cezo *et al.*, 2014) proposed use of the breaking strength and shear stress as supplements to burst pressure. However, all these methods do not assess fusion strength without disrupting the anastomosis.

Bio-impedance of tissue is an electrical frequency-dependent characteristic, which is found rising during heating process (Arya *et al.*, 2013) due to intracellular fluid evaporation and cells rupture. For this reason, it has been applied in feedback control loops in vessel thermal sealing device (Kennedy *et al.*, 1998). However, the present study demonstrated only a weak negative correlation between the absolute bio-impedance and fusion strength at frequency of 445 kHz. Hence the results of the present study suggest that absolute bio-impedance is unlikely to be reliable in identifying end of the tissue fusion process. The limitations of bio-impedance feedback have been articulated by Floume as 'significance bio-impedance changes might be expected to occur only in the final stage of RF delivery when tissue water boils' (Floume *et al.*, 2008; Floume, 2008; Floume *et al.*, 2010). Arya (Arya *et al.*, 2013) proposed using the rise in relative impedance to 200 $\Omega$  to detect the end of fusion process, but the exact mechanism for this remains unclear.

In contrast, the present study demonstrated a strong highly significant negative correlation between reactance modulus and burst pressures at frequency of 445 kHz, indicating that the mean reactance modulus can be distinguished between burst pressure levels and has the potential to identify the end of the fusion process. We attribute the descending of reactance modulus as being caused by the rise in capacitance, consequent on cells shrinkage. The outer boundary of cell (plasma membrane) consists largely of phospholipid molecules, which become a dielectric, and behave as a membrane capacitor (Schwan, 1994). When current flows into tissue, cells shrink due to loss of intracellular fluid by evaporation (Floume, 2008). The present study documented decreased reactance modulus after thermo-fusion, inferring that the fusion strength is indirectly related to the extent of cells shrinkage. In line with this observation, the study demonstrated concordance, i.e., low burst pressures are accompanied by diminished cells shrinkage, reflected by a high reactance. It is possible that cells shrinkage also trigger a series of tissue interactions during tissue fusion leading to collagen crosslinking. Hence, reactance offers a qualitative index of the fusion strength.

Reactance because of its relation to burst pressures merits inclusion in closed loop systems based on the basis of present study and previous study reported by Gonzalez-Correa (Gonzalez-Correa *et al.*, 2005) which demonstrated that bio-impedance increases with rising compression pressure. In addition, factor that ratio of probe to electrode size should be considered as well (Hajjari *et al.*, 2010).

Progress in the technology of RF-induced tissue fusion for colorectal and other GI anastomosis will accelerate if an effective control closed loop is developed which will automatically halt the tissue fusion before the onset of thermal damage to the fused seal. The present study has demonstrated that reactance, which is the component of bio-impedance reflecting cells shrinkage is likely to be a key component of the feedback loop designed to enhance the strength, reliability and safety of RF-induced tissue fusion. Nevertheless, we acknowledge some limitations of present study. Ischemia is an inevitable limitation in ex-vivo experiment. However, the influence of intestine ischemia seems unclear. As researches indicate the sensitive frequency for cerebral ischemia monitoring is 6kHz or 8kHz, we temporarily assume the results are not affected considerably by ischemia because the frequency of electrosurgical devices is much higher than that (Hug and Haag, 2011).

So far, the nonlinear effects of impedance caused by hyperthermia are not involved because frequency-

domain bio-impedance of fused colon are studied instead of real-time heating process. Thus, further research regarding time-domain bio-impedance characteristics affected by temperature is necessary. Moreover, whether more sensitive detecting frequency exists is deserved investigation.

**Acknowledgments**

The authors thank help of Mr. Dick Lee at Covidien Centers of Innovation in Shanghai. This work was supported by the National Natural Science Foundation of China (No. 51377024), China Scholarship Council, Science and Technology Commission of Shanghai Municipality (No. 13441900802) and the Natural Science Foundation of Shanghai (No. 14ZR1428300).

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